Energy and Carbohydrate Metabolism in Magnesium-deficient Calves

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Magnesium participates in almost all phosphorylating enzyme systems of the animal body, and it is to be expected therefore that when animals are deprived of dietary magnesium abnormalities of intermediary energy transfer would result. Indeed, some of the signs of Mg deficiency are similar to those of thiamine deficiency, in which abnormalities of carbohydrate metabolism are known to occur. The present paper is concerned with the total energy exchange and certain aspects of carbohydrate metabolism of calves given rations deficient in Mg. The results show that the elevation of heat production and the abnormalities of carbohydrate metabolism that were observed were not due to failures of enzyme systems in which Mg acts as an activator, but to an increase in muscular activity.

EXPERIMENTAL

Animals. Ten bull calves were used. Two pairs each consisting of a control (nos. 214 and 219) and a Mg-deficient animal (nos. 215 and 220) were used in studies of total energy metabolism throughout the whole course of production of the Mg-deficiency syndrome. These involved 108 determinations of the 24 h exchange of energy, water, carbon and nitrogen. One pair was the subject of experiment from September to December 1952, the other from January to April 1953. The experiments with the remaining six calves took place from September to December 1953. Two (nos. 225 and 226) were given the control diet, two (nos. 221 and 222) the diet deficient in Mg and two (nos. 223 and 224) a diet deficient in thiamine, and their carbohydrate metabolism was studied. The general treatment of the calves was as previously described (Blaxter, Rook & MacDonald, 1954).

Diets. The normal diet and the Mg-free diet described by Blaxter, Rook & MacDonald (1954) were used. The thiamine-free diet was obtained by replacing the commercial casein of the normal diet with vitamin-free casein, by omitting thiamine from the supplement and replacing the liver extract with a supplement of vitamin B_{12} and folic acid. All calves were given 4 l. daily of the control diet for a short while before the experiment began. Of the experimental diet, 4 l. daily were given, increasing to 6 l.

Methods. Energy exchange was determined in a closed-circuit respiration apparatus (Blaxter, Graham & Rook, 1954). Calorific values were determined by bomb calorimetry, carbon by direct combustion and nitrogen by the Kjeldahl procedure. The factors of Blaxter & Rook (1953) were used to estimate fat retention from the carbon and nitrogen balances.

Blood glucose was determined by the method of Nelson (1944), pyruvic acid in whole blood and urine by the method of Friedemann & Haugen (1943) and lactic acid by that of Barker & Summerson (1941). Alkaline-phosphatase activity in blood serum was determined by a modification of the method given by Hawk, Oser & Summerson (1947) using disodiumphenylphosphate as substrate in veronal buffer. Serum calcium and Mg were determined by the method of Godden (1935). Thiamine was determined in urine and digesta by the method of Mickelsen, Condiff & Keys (1945). Venous blood samples for routine estimations were taken at weekly intervals 4 h after feeding. Collections of 24 h samples of urine were made twice weekly. Glucose-tolerance tests were made after the intravenous injection of 30–40 g glucose (approx. 1 g/kg body-weight). Exercise-tolerance tests were made after 3–7 min of exercise (running and walking), initial values being obtained after restricted movement for at least 12 h.

Clinical observations were made daily: detailed post-mortem dissections were also made.

RESULTS

Heat production during the deficiency. Studies with rats by Kleiber, Boelter & Greenberg (1941), and by Kleiber (1945–6) showed that experimental magnesium deficiency results in an almost immediate increase in heat production. This was not so with the calves. Heat production estimated from the oxygen consumption and non-protein respiratory quotient remained unaffected until gross signs of tetany were apparent. The carbon-dioxide production and oxygen consumption of both pairs of calves are given in Table 1. In Fig. 1 the heat production computed from the oxygen consumption, carbon-dioxide production and urinary nitrogen excretion is given in detail for one pair, and in Fig. 2 the carbon-dioxide production is given in detail for the other pair. It will be noted that the increased heat production, oxygen consumption and carbon-dioxide production were correlated with the fall in the Mg concentration

Table 1. *Mean values for CO_2 production, O_2 consumption and Mg content of the serum of control and Mg-deficient calves given 6 l. daily of the liquid diet of Blaxter, Rook & MacDonald* (1954)

		Control calves			Magnesium-deficient calves		
Pair no.	No. of days on deficient ration	Serum Mg (mg/100 ml.)	CO_2 production (l./24 h)	O_2 consumption (l./24 h)	Serum Mg (mg/100 ml.)	CO_2 production (l./24 h)	O_2 consumption (l./24 h)
1*	18–21	2·05	352·7	443·6	0·73	350·3	443·1
	36–39	2·07	405·9	505·9	0·35†	482·5	623·3
	40–46	2·00	397·6	508·1	0·49†	483·6	640·9
2‡	25–32	2·46	433·0	541·2	1·10	441·0	554·9
	49–54	2·33	460·0	581·4	0·74	457·1	583·8
	61–64	2·23	459·9	599·8	0·62†	496·2	641·9
	65–67	2·45	456·1	593·9	0·53†	505·0	671·7

* The control calf received a diet containing 12 mg Mg/100 ml., and the Mg-deficient animal one containing 0·4 mg/100 ml.

† Tetany present.

‡ The control calf received a diet containing 19 mg Mg/100 ml., and the Mg-deficient animal one containing 0·4 mg/100 ml.

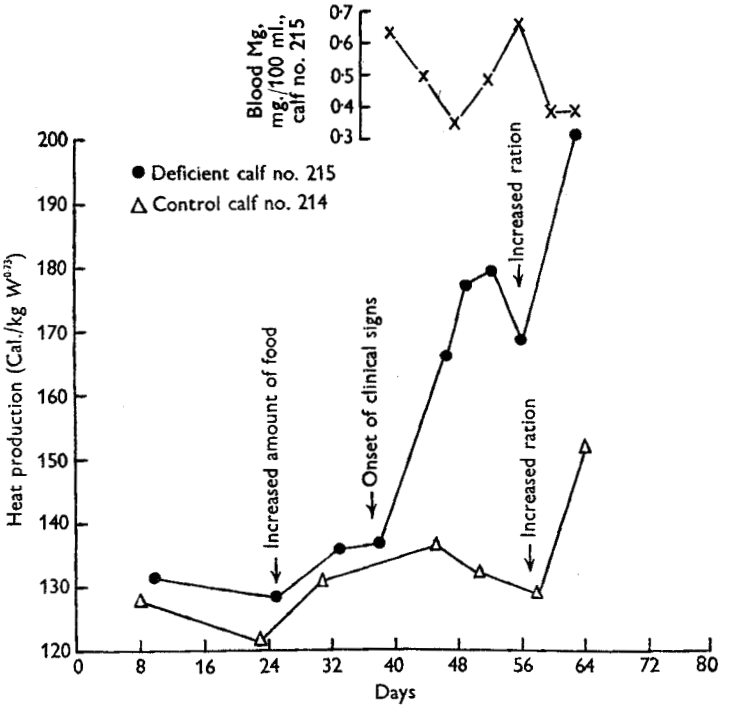

Fig. 1. The effect of Mg deficiency on heat production, and the blood-serum Mg concentration in calves nos. 214 and 215.

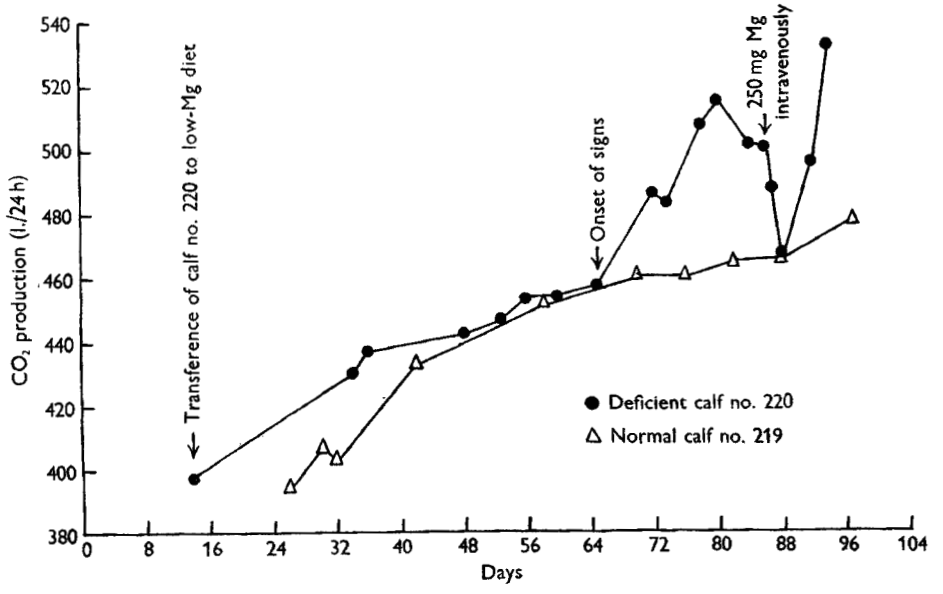

Fig. 2. The acceleration of CO_2 production by Mg deficiency and its abolition by the intravenous injection of 250 mg Mg as the sulphate, calves nos. 219 and 220.

in the blood serum. With calf no. 215, a slight rise in blood-serum Mg of unknown cause was associated with a fall in heat production. The intravenous injection of 250 mg Mg as the sulphate reduced the heat production of calf no. 220 to a value comparable to that observed in the control animal given the same quantity of diet. The effect was not very prolonged and tetany reappeared within 3 days, when the blood-serum concentration once more fell to 0·5 mg/100 ml. No abnormalities of the respiratory quotient were noted. There was a slight increase in the evaporative loss of heat from the deficient calves, but only when tetany was present.

Table 2. *Mean daily retention of carbon, nitrogen and energy by control and Mg-deficient calves during the course of the experiment*

Calf no.	Body-weight (kg)	Energy intake (Cal.)	Nitrogen (g)				Carbon (g)					Energy retention (Cal.)
			Excretion			Body storage	Excretion				Body storage	
			Intake	Faeces	Urine		Intake	Faeces	Urine	Respiration		
Pair 1 before clinical signs developed, 4 l. diet												
214 (control)	38·4	2474	21·2	1·6	14·9	+4·7	214·5	12·7	12·4	160·6	+29·9	+387
215 (deficient)	36·9	2551	19·7	2·3	15·4	+2·0	221·0	19·6	11·6	164·5	+25·4	+305
Pair 1 before clinical signs developed, 6 l. diet												
214 (control)	50·2	3790	32·3	1·0	20·3	+11·0	326·1	8·5	18·8	212·0	+86·8	+1013
215 (deficient)	44·2	3997	31·6	3·0	17·4	+11·2	323·9	17·6	24·7	203·3	+78·3	+905
Pair 1, clinical signs present in calf no. 215, 6 l. diet												
214 (control)	54·7	3948	32·3	0·9	21·6	+9·8	311·5	8·5	19·3	214·4	+69·2	+800
215 (deficient)	48·2	3818	31·7	4·6	25·7	+1·4	283·5	23·9	26·1	260·8	−27·3	−352
Pair 2 before clinical signs developed, 4 l. diet												
219 (control)	37·6	2629	22·0	1·4	14·0	+6·6	234·4	10·8	15·1	154·4	+54·1	+634
220 (deficient)	34·5	2667	20·3	2·6	14·0	+3·7	237·9	12·6	14·3	164·3	+46·8	+562
Pair 2 before clinical signs developed, 6 l. diet												
219 (control)	41·9	4047	32·8	1·2	17·0	+14·6	352·7	9·1	18·7	219·0	+105·8	+1228
220 (deficient)	49·5	4187	33·8	2·1	17·1	+14·6	370·3	14·2	17·2	231·8	+107·0	+1242
Pair 2, mild clinical signs present in calf no. 220, 6 l. diet												
219 (control)	66·0	3956	33·0	0	20·3	+12·7	322·3	0	22·5	247·9	+51·8	+563
220 (deficient)	64·9	4094	32·4	1·3	19·0	+12·1	327·1	8·0	17·8	261·2	+40·1	+420
Pair 2, clinical signs present in calf no. 220, 4 l. diet												
219 (control)	64·2	2774	21·3	0·7	16·4	+2·2	219·2	4·3	16·0	233·2	−34·3	−247
220 (deficient)	66·6	2779	22·2	1·6	20·1	+0·5	235·5	9·1	16·1	264·2	−53·9	−680

Energy retention and the specific dynamic effect of food. Typical energy balances of the calves during both the pre-tetany and tetanic phases of the deficiency are given in Table 2. There were relatively small differences in energy utilization in the pre-tetany phase. During tetany, however, energy retention fell, largely owing to the decrease in fat retention associated with increased respiratory loss of carbon dioxide. The differences in nitrogen metabolism possibly reflect this change in energy expenditure. In Fig. 3 the heat production of the calves has been plotted against their food intake expressed as metabolizable energy. Following the approach of Marston (1948) both values have been scaled by dividing by body-weight raised to the power 0·73. This

procedure effectively allows for differences in body size. It will be noted that the Mg-deficient calves lost more heat at comparable intakes of metabolizable energy. Conventionally, the fact that the two lines relating heat production to metabolizable energy are approximately parallel might be regarded as implying that the difference in efficiency results from a difference in basal metabolism. Thus the difference in heat production or, conversely, energy retention, would still exist on extrapolation to a food intake of zero. In fact, the rise in heat production represents an increased muscular activity and the important point is that there is no acceleration of heat loss at high levels of feeding. If the terminal phase of the deficiency was associated with abnormalities of intermediary energy metabolism, and the additional heat loss was due to

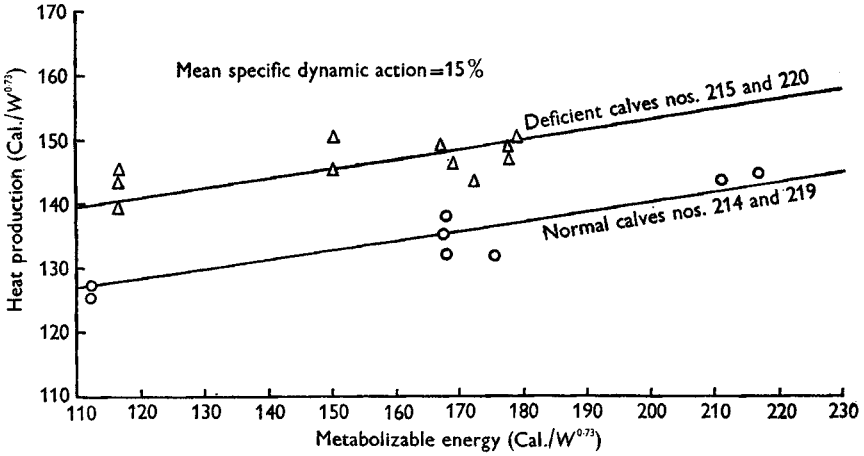

Fig. 3. The effect of nutritional plane on heat production/kg metabolic body size in normal and Mg-deficient calves.

failure of Mg-activated enzyme systems to deal with the intrinsic free energy of the products of digestion, then heat losses would presumably increase with increasing food intake or, alternatively, appetite would fail. The latter did not occur. It may be concluded, therefore, that the studies of the total energy exchange of the Mg-deficient calf do not reveal any gross abnormality in the metabolic pathways of food dissimilation and anabolism.

Carbohydrate metabolism. It is quite possible, however, that Mg deficiency might lead to abnormalities of metabolism that would remain undetected by the gross methods of study outlined above, by virtue of the fact that alternative katabolic pathways exist. Besides acting as an activator of phosphatases, Mg is always required in enzyme systems involving thiamine pyrophosphate. In fact the whole chain of reactions whereby carbohydrate is dissimilated requires the participation of Mg as an activator or a part of the enzyme-coenzyme complexes. The primary phosphorylation of glucose, the formation of the 1:6-phosphate as well as the dissimilation of pyruvate require the presence of Mg ions. Furthermore, in the breakdown of adenosine triphosphate to adenosine diphosphate to provide energy for muscular contraction, the enzyme concerned, though activated by Ca ions, is inhibited by Mg ions. The chemical

determinations and functional tests made on thiamine-deficient, Mg-deficient and normal calves were designed to test whether or not these deficiencies resulted in comparable abnormalities of metabolism.

Blood glucose and glucose tolerance. The concentration of glucose in the blood varied in all calves between extremes of 50 and 110 mg/100 ml. The variation was related to the incidence of slight diarrhoea, and the trend of the values with time revealed no differences between the groups. Terminally when both Mg-deficient calves were in tetany the blood glucose values were 87 and 90 mg/100 ml. The values in control calves at this time were 109 and 73 mg/100 ml. The final value in the one calf which succumbed to thiamine deficiency was 79 mg/100 ml.

Table 3. *The constant τ of exponential equations relating blood sugar concentration to time following injection of glucose in normal, Mg-deficient and thiamine-deficient calves*

Group	Calf no.	τ (min)
Control	225	33·1*
	226	45·1
Magnesium-deficient	221	43·9
	222	36·3
Thiamine-deficient	223	38·8
	224	47·6
	224 (signs of thiamine deficiency present)	58·2

* Based on results of one determination only, remainder based on results of two determinations.

Glucose-tolerance curves obtained following the intravenous injection of 30 g glucose were analysed in the following way. The rate of removal of glucose from the blood is nearly exponential. The time constants τ of exponential equations relating blood glucose to time were therefore obtained and are given in Table 3. They show no difference in the rate of disappearance of excess glucose as between calves deficient in Mg and control calves receiving ample. The fact that no abnormal rise was noted in the blood pyruvate or lactate following the injection of sugar suggests that the subsequent metabolic fate of the sugar followed a normal path. No clinical signs of abnormality were noted in Mg-deficient calves when up to 80 g glucose were given intravenously.

Blood pyruvic acid and lactic acid and urinary pyruvic acid. In Figs. 4 and 5 the mean values for blood pyruvic acid and blood lactic acid in the Mg-deficient and control calves are given. The variation in the concentration of these substances in the blood from time to time was not small and was associated again with alimentary disturbance, low values coinciding with short bouts of diarrhoea. At these times the fall was most marked in lactic acid, and was due apparently to a reduction of the effective food intake. Nevertheless, the onset of tetany in the calves was associated with a rise in the pyrucic acid and lactic acid in the blood. In Table 4, the mean daily amounts of pyruvic acid excreted in the urine are presented. It will be noted that the increased quantities of pyruvic acid in the blood during tetany were reflected in the urinary excretion.

Thiamine deficiency. The above results suggest that lactic acid and pyruvic acid accumulate in the blood when the tetanic phase of Mg deficiency is reached and that pyruvic acid is excreted in the urine in consequence. These results would suggest that this phase of the disease is comparable to thiamine deficiency. Also, opisthotonos is a characteristic clinical sign in the early stages of Mg deficiency (Blaxter, Rook & MacDonald, 1954) as it is in thiamine deficiency in calves (Johnson, Hamilton, Nevens & Boley, 1948) and lambs (Draper & Johnson, 1951).

Table 4. *Mean daily excretion of pyruvic acid in the urine by normal, Mg-deficient and thiamine-deficient calves, expressed in mg/day*

	Replicate 1	Replicate 2
Control calves nos. 225 and 226*	35·0 ± 4·3	38·6 ± 3·8
Mg-deficient calves nos. 221 and 222:		
Before appearance of clinical signs	37·0	39·0
During tetany	65·0	52·0 – 56·0
Thiamine-deficient calf no. 224 (clinical signs present)		73·0 – 86·0
Thiamine-deficient calf no. 223:		
Very slight clinical signs present	55·0 – 67·0	
Following sulphonamide and streptomycin therapy, no clinical signs	82·0 falling to 47·0	

* Value with standard deviation obtained from the series of weekly estimations.

Table 5. *Thiamine excretion (μg/day) in the urine by normal and thiamine-deficient calves*

	Control calves		Thiamine-deficient calves	
Day	No. 225	No. 226	No. 223	No. 224
5	159	0	114	81
12	180	132	48	0
19	282	696	0	0
26	678	621	0	0
33	312	270	30	1
40	480	459	39	1
47	1095	1011	48	0
54	3558	2664	10	0
61	2287	1666	115	0
68	5012	1645	101	0
75	3605	3499	81	0

Of the two calves given the thiamine-deficient diet one showed severe clinical signs of deficiency and eventually was killed *in extremis*. The other showed mild signs after a few weeks, but these regressed. Large doses of penicillin, streptomycin, phthalysulphathiazole or sulphanilamide failed to provoke clinical signs, and the calf remained healthy. The urinary excretion of thiamine in control calves and those deficient in thiamine is shown in Table 5. It will be noted that the urinary thiamine, after falling to a very low level, increased markedly after a month in calf no. 223, and was not reduced to zero by antibiotic and other therapy. A thiamine balance of this calf is given in Table 6.

Clearly, synthesis of thiamine was taking place in the gut contents. The calf was killed and thiamine in the gut contents was determined. The results expressed as

μg thiamine/100 g digesta were: rumen 59·6, abomasum 80·4, small intestine 70·8, caecum 113·4 and the remainder of the large intestine 213·8. The dietary concentration was 3·0 μg/100 g and clearly massive synthesis was taking place. In all samples of digesta hair was present which, despite daily grooming, the calf had obtained by licking itself.

Table 6. *Intake and excretion of thiamine by thiamine-deficient calf no.* 223 (*66th day of experiment*) *expressed as* μg/24 *h*

Intake	24
Faecal excretion	316
Urinary excretion	81
Minimal estimate of synthesis in the gut	373

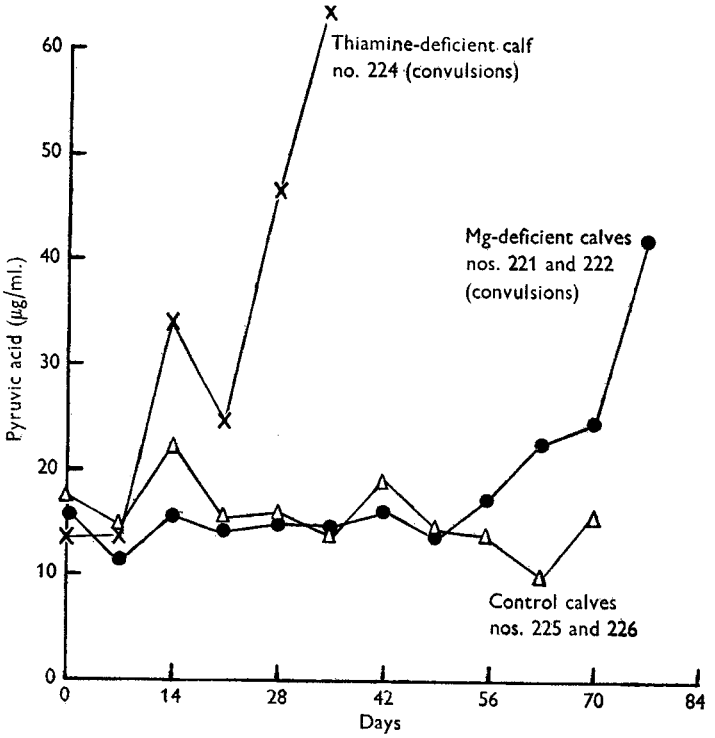

Fig. 4. The mean concentration of pyruvic acid in the blood of control calves, Mg-deficient calves and thiamine-deficient calf no. 224.

The clinical signs in calf no. 224 were severe opisthotonos, paraplegia, anorexia, ataxia and prostration. There was no tetany. From Figs. 4 and 5 and from Table 4, it is evident that thiamine deficiency in calf no. 224 had profound effects on pyruvic-acid and lactic-acid metabolism. The effect on pyruvic acid was particularly marked, the elevation in blood pyruvate being far greater than in Mg deficiency. These effects are comparable to those of thiamine deficiency in other species.

Post-mortem examination of calf no. 224 revealed no gross lesion other than haemorrhage into the meninges of the brain. Histological examination of the tissues of the calf showed no lesion save fatty changes in the liver. These changes may have

been due to terminal anorexia and inappetence, though limited amounts of food were in fact given by stomach tube when this phase of the deficiency was reached.

Anoxia in Mg deficiency. Our previous experiments showed that in most Mg-deficient calves in the tetanic stage, body temperature was elevated. In one calf, for instance, which survived a convulsive attack, body temperature rose from 101·7 to 105·4° F in 15 min. The respiratory frequency became almost uncountable, the pulse rate was close to 200/min and the cutaneous vessels became grossly dilated. It may be calculated from the body temperature change that during the convulsions in this calf, heat production increased at least fourfold. Such muscular work must involve anaerobic dissimilation of pyruvate and the accumulation of an oxygen debt. Exercise-tolerance tests were made with control calves and calves showing the first signs of tetany. Typical results are given in Fig. 6, for a calf showing clinical signs of Mg deficiency and for a control calf.

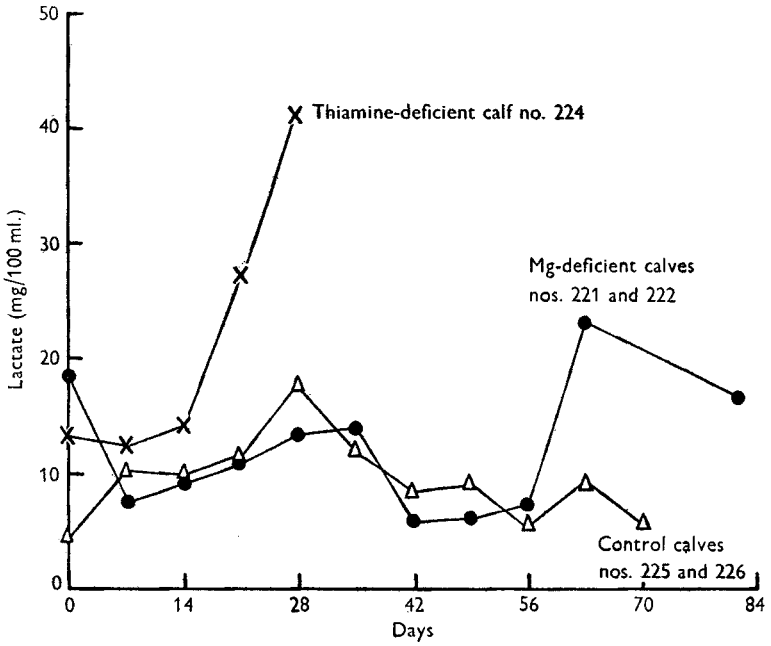

Fig. 5. The mean concentration of lactic acid in the blood of control calves, Mg-deficient calves and thiamine-deficient calf no. 224.

Exercise elevated the body temperature, the pulse rate, the respiratory frequency and the concentrations of lactic and pyruvic acids in the blood. In four tests with control calves the blood pyruvic-acid levels observed at the end of exercise were 32, 60, 42 and 60 μg/ml., higher than the values found in resting Mg-deficient calves but lower than the value obtained in the terminal phase of thiamine deficiency. With Mg-deficient calves exercise resulted in increases in temperature, pulse and respiration and in the blood concentration of pyruvic acid and lactic acid comparable to those found in control calves under similar conditions. This would suggest that the increase in blood pyruvate and lactate and in urinary pyruvate excretion observed in

Mg-deficient calves in the tetanic stage was contingent upon the muscular activity of tetany, rather than upon partial failure of Mg-activated enzyme systems to remove pyruvic acid from the cells, and this would account for the absence of any abnormality in pyruvate metabolism following administration of sugar. Furthermore, it may be seen from Fig. 6 that following exercise the pyruvic-acid and lactic-acid concentration in the blood fell at comparable rates. This finding supports the conclusion that there is no serious abnormality of pyruvate and lactate metabolism in Mg-deficient calves.

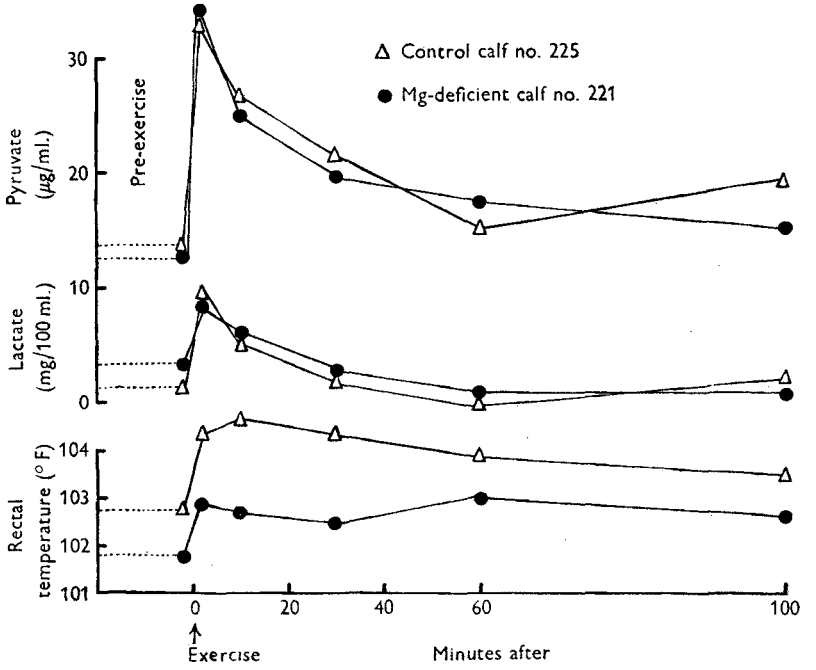

Fig. 6. The effect of 3–7 min exercise on the rectal temperature, and the concentration of pyruvic acid and lactic acid in the blood of a normal and a Mg-deficient calf. The Mg-deficient calf had shown severe tetany previously but was only hyperexcitable when the test was made.

Table 7. *Alkaline-phosphatase activity of the blood serum of normal and Mg-deficient calves (phenol units: μg/phosphate liberated/ml. serum)*

Calf no.	Value before treatment	Value during tetany in Mg-deficient animals	Increase in activity on addition of 1 ml. 0·03 M-$MgCl_2$ to 1 ml. serum
	Controls		
225	139	134·0	11
226	87	102·5	0
	Mg-deficient		
221	66	60·5	2
222	63	94·0	4

Serum phosphatase activity. In Table 7, the salient features of the results of analyses of the serum for phosphatase activity are given. It will be noted that there was no significant change in phosphatase activity even though the serum Mg was

reduced to a level at which tetany occurred. Furthermore, the addition of $MgCl_2$ (1 ml. of 0·03 M solution/ml. serum) did not result in any significant activation of the serum phosphatase in these calves. It may be concluded therefore that in tetanic calves the ionized moiety of the serum Mg or at least that fraction available for the activation of alkaline phosphomonoesterases is not sufficiently depressed to affect the enzyme studied.

DISCUSSION

The above results show that not only is Mg deficiency associated with no abnormality of the heat increment of feeding (the specific dynamic effect), but that no abnormality can be detected in the carbohydrate metabolism of the animal that might be related to the known essentiality of Mg as an activator of a number of enzyme systems. The accumulation of pyruvic and lactic acid may be explained by the increased muscular work performed in tetany, a conclusion supported by the measurements of heat production, and also by records of the performance of normal and deficient calves following exercise. The direct comparison of the effects of thiamine deficiency with those of Mg deficiency are in agreement with this contention.

Further confirmatory evidence is that the increased metabolic load induced by the intravenous injection of glucose did not result in any exacerbation of clinical signs and that the concentration of Mg in the soft tissues and organs of deficient calves if it is reduced at all is reduced by only a very small fraction (Blaxter, Rook & MacDonald, 1954).

The clinical sign of opisthotonos in Mg deficiency in the calf remains to be explained. Opisthotonos in the magnesium-deficient calves was never so severe as either that noted in calf no. 224, which succumbed to thiamine deficiency, or that shown in thiamine-deficient animals, including lambs. It is possible that opisthotonos is a reaction to abnormally high or maintained concentrations of pyruvate in the blood or tissues particularly the blood supplying the central nervous system. Thus Dobrovolskaia-Zavadskaia (1946) found that injection of pyruvate into mice resulted in opisthotonos, and Draper & Johnson (1951) found retraction of the head and convulsions to follow its injection into lambs. As previously reported (Blaxter, Rook & MacDonald, 1954), opisthotonos in Mg deficiency is not associated with any demonstrable histological abnormality in the brain. Part, at least, of the apparent central nervous involvement may be due to metabolite accumulation as a result of an accelerated anaerobic muscular metabolism.

SUMMARY

1. Magnesium deficiency was produced in four calves and thiamine deficiency in two calves and the energy exchange and carbohydrate metabolism were studied. Four control calves were used.

2. Mg deficiency resulted in an increase in heat production and a fall in energy retention, the magnitude of these effects being related to the serum concentration of magnesium. Injection of Mg sulphate abolished them.

3. The increase in heat production was not due to an increase in the specific dynamic action, but was associated with increased muscular activity.

4. The concentration of glucose in the blood and the tolerance to glucose injected intravenously were unaffected in Mg deficiency.

5. An increase in the concentration of pyruvic and lactic acids in the blood occurred when clinical signs of Mg deficiency were present. An increased urinary excretion of pyruvic acid also took place at these times.

6. Thiamine deficiency was associated with a much greater increase in the pyruvic-acid content of the blood than in Mg deficiency. Evidence of considerable synthesis of thiamine in the alimentary tract was obtained with one calf.

7. Exercise-tolerance tests showed that in normal animals the concentration of pyruvic and lactic acids in the blood was increased to levels comparable to those found in resting Mg-deficient calves exhibiting mild tetany. It was concluded from this and other evidence that the abnormalities of energy and carbohydrate metabolism observed in Mg deficiency were due to the increased muscular work of tetany and not to any effect of lack of dietary Mg on enzyme systems concerned in carbohydrate metabolism.

8. Mg deficiency even in the final phases had no effect on the activity of the serum phosphatase of the calves.

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